Footprints of the Mediterranean Sea history on the molecular variation of mtDNA and introns in the clam *Ruditapes decussatus*

David Cordero, Amaya Velasco, Juan B. Peña, Carlos Saavedra

Instituto de Acuicultura de Torre la Sal, Consejo Superior de Investigaciones Científicas, Ribera de Cabanes, 12595 Castellón, Spain.
Email:Saavedra@iats.csic.es

The carpet-shell clam *Ruditapes decussatus* (family Veneridae) is a bivalve mollusk of great interest in fisheries and aquaculture. It lives buried in sandy bottoms of the intertidal zone in the Mediterranean Sea and adjacent Atlantic regions. The life cycle is characterized by a planktonic larval phase of 2-3 weeks and a sessile adult stage that can last for several years.

Some of the carpet-shell clam stocks are experiencing overfishing. The introduction of exotic species such as the manila clam *R. philippinarum* is also a pressure on *R. decussatus* populations since they compete for space and resources. Due to aquaculture, an extensive mixing of stocks at some localities could be taking place, with unpredictable consequences to the integrity of the species' genetic diversity. For all these reasons the study of the genetic structure of carpet-shell clam is of great interest.

Previous studies on the carpet-shell clam showed low allozyme differentiation of Atlantic and Mediterranean populations [1] [2] [3], but were based on few populations and loci. Furthermore, these results were in contrast with other studies of genetic differentiation of marine organisms of the Mediterranean and northeast Atlantic waters. These studies usually showed important differentiation associated with the main marine basins, which was explained as a result of the recent geological and oceanographic history of the region [4]. On the grounds of these considerations, we have started a study of carpet-shell clam population genetics. Our first results based on the study of length polymorphisms at 3 nuclear markers revealed a high genetic differentiation between two populations of the Atlantic and the Mediterranean [5]. The interest of these results encouraged us to continue the work in this direction. In this poster we present new data based on the study of the molecular variability of a mitochondrial marker (COI) and 6 intronic markers in 11 carpet-shell clam populations spread over the natural distribution range of the species.

Fig. 1 Populations of carpet-shell clam sampled in this study.

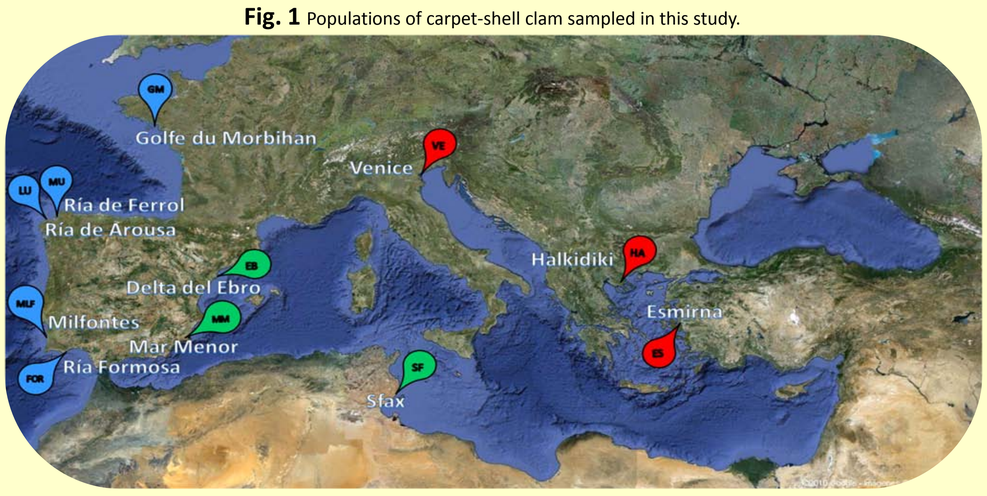
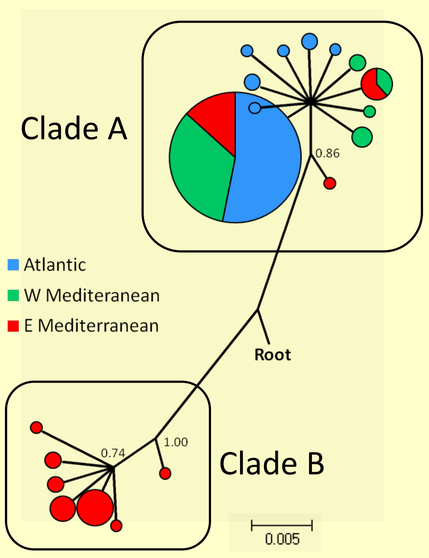


Fig. 2 Bayesian phylogenetic tree of haplotypes found at the COI gene fragment.



Analysis with genetic markers

Six nuclear markers as well as sequencing of a 459 base-pairs-long fragment of the mitochondrial COI gene were used for the study of genetic diversity and differentiation of clam populations.

-Mitochondrial DNA
The COI phylogeny showed the existence of two main clades (A and B) with a divergence of 2 %. These clades have a different geographic distribution. Clade A is present in all the samples and clade B appears solely in the Aegean Sea populations.

-Intron markers
The polymorphisms of 6 intron markers were studied by means of RFLPs (Fig. 3). A length polymorphism was studied in an additional marker [5]. No polymorphism was found at marker CL10iA. The remaining markers were found to have 2 to 4 allelic variants which, in the case of RFLP markers, were detected by only one restriction enzyme. Most alleles were found all over the sampled area, but some alleles appearing at moderate frequencies at two loci (CL126iA and TBP) were restricted to the east Mediterranean basin.

The AMOVA [6] of genic frequencies (Fig. 4) revealed an important genetic differentiation among the Atlantic, west and east Mediterranean basins in three of these markers (CL126iA, CL116iA, TBP). An additional Bayesian analysis [7] confirmed these results (Fig. 5). Locus CL102iA , despite of a high FST value (0.216) exhibited a low genetic differentiation among basins (Fig. 5).

A paleogeographic model to explain the results

Former studies with similar results in other Mediterranean species have been explained on the basis of sea level changes and fragmentation of the Mediterranean basins that occurred during Pleistocene (3 MY ago to present). During the last glacial maximum the sea level dropped about 150 m, which resulted in the isolation of the Black Sea basin, the restriction of water movement among Atlantic, west, and east Mediterranean basins, and isolation of the Aegean Sea. These phenomena would have enabled the isolation of the populations of marine organisms, conducing to genetic differentiation [4] [8] [9].

Our results can be explained by these models. The distribution of the two mitochondrial clades would be the consequence of the Aegean Sea isolation during the glacial maximum. Studies in other species such as the cockle *Cerastoderma glaucum* [9] found a similar phylogeographic pattern at mitochondrial DNA. Parallelism between different species supports the paleogeographic hypothesis.

The strong genetic differentiation observed among basins at nuclear markers also fits this model. Existing alleles with intermediate frequencies at loci CL126iA and TBP, which are restricted to the east Mediterranean basin, may be the result of the isolation during the Pleistocene.

In order to test this model we need to increase our resolving power. Therefore we have started the analysis of the intron sequences.

Fig. 3 RFLP detection of intronic markers in the carpet-shell clam. Primers flanking introns were designed from ESTs derived from a cDNA library [11]. PCR products were digested with a restriction enzyme (Dra I or BamH I) and fragments separated by electrophoresis in 1.5-2 % agarose gels.

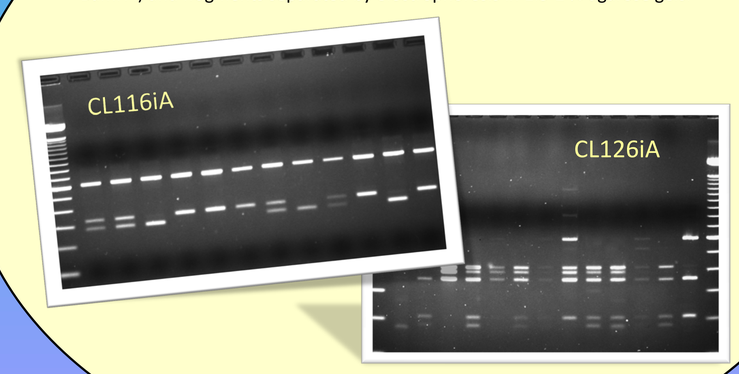


Fig. 4 Results of the hierarchical F-statistics analysis of the intronic markers. Populations were pooled according to basins (Atlantic, West Mediterranean and East Mediterranean). The total genetic variance FST was partitioned into within-basin (FSC) and among-basin (FST) components .

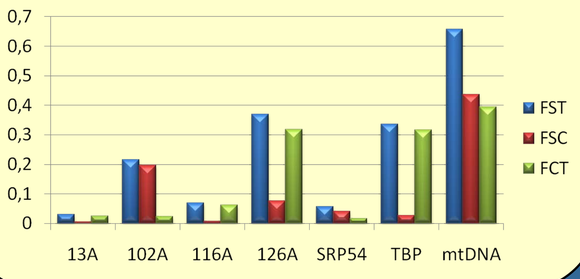


Fig. 5 Bayesian cluster analysis carried out with STRUCTURE on 11 clam populations under the models of admixture and correlated allele frequencies for a value of k=3. Three clusters of clear geographic distribution are apparent.

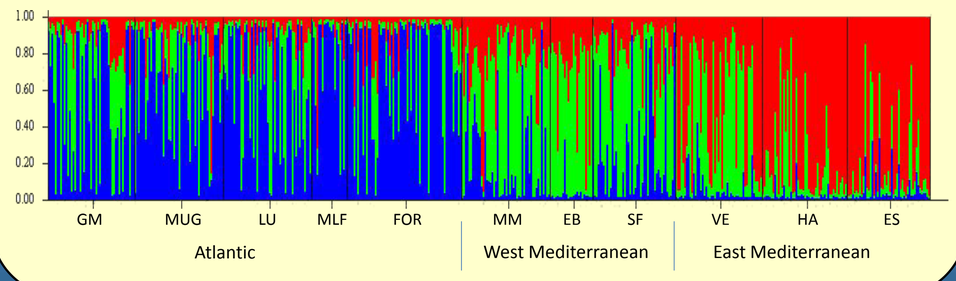


Fig. 6 Haplotype network of locus **CL10iA**. The circle size is proportional to the haplotype frequency.

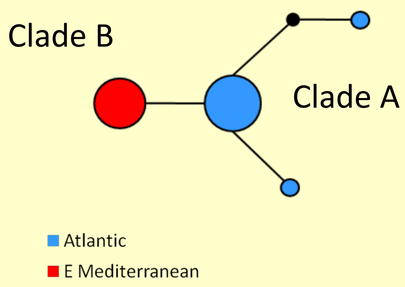


Fig. 7 Bayesian phylogenetic tree of haplotypes found at locus **CL126iA**. Colors as in Fig. 6.

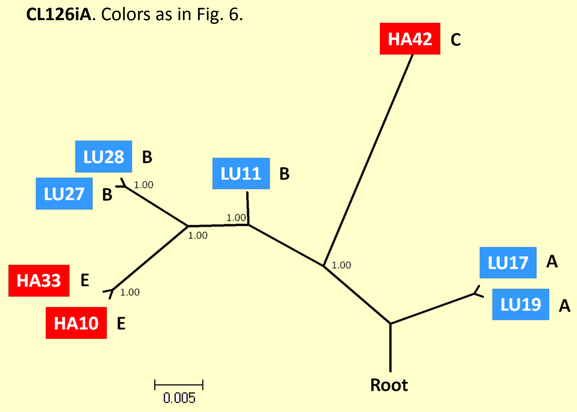


Fig. 8 Bayesian phylogenetic tree of haplotypes found at locus **CL116iA**. Colors as in Fig. 6. The circle size is proportional to the haplotype frequency. Letter beside circles refer to RFLP allelic designations.

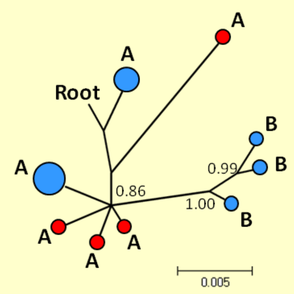


Fig. 9 Bayesian phylogenetic tree of haplotypes found at locus **CL13iA**. Details as in Fig. 8.

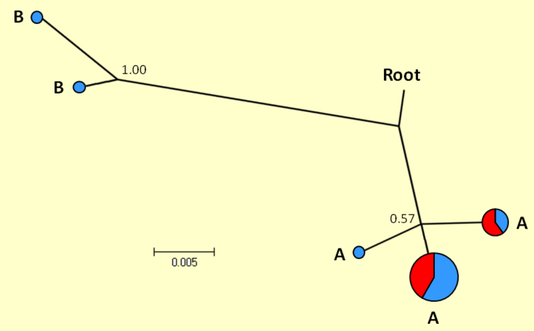
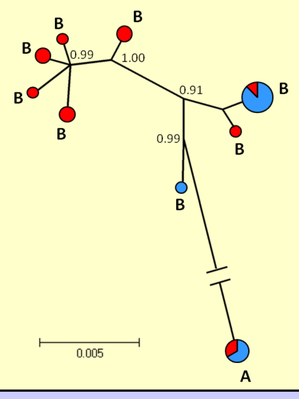


Fig. 10 Bayesian phylogenetic tree of haplotypes found at locus **CL102iA**. Details as in Fig. 8.



Population analysis of intronic sequences

Five markers were sequenced in 10 individuals from two very distant populations, one from the Atlantic (LU) and another from the east Mediterranean (HA), in order to have a preliminary evaluation of intra as well as interpopulation diversity. We found that all markers showed more diversity than observed with RFLPs. We also found a corroboration of the paleogeographic hypothesis in three out of the five markers and new details of the process of genetic differentiation of clams were ascertained.

The most striking case was marker CL10iA, which had no RFLP variation but after sequencing showed alternative fixed haplotype sets (A and B) in each population which differed in only one nucleotide site (Fig. 6). Similarly, specific eastern Mediterranean clades were detected in all other markers except CL13iA, the locus that presented the lowest FST values in the study of RFLPs (Fig. 7-10). Sequencing has also discovered that some common RFLP alleles that have a wide geographic distribution have cryptic variants that show a geographic association (allele A of locus CL116iA and allele B of locus CL102iA) (Figs. 8 and 10). It also showed that moderate frequency alleles restricted to the eastern Mediterranean in the locus CL126iA form separate clades (Fig. 7).

All these observations suggest that the eastern Mediterranean clam populations underwent a period of isolation during which new haplotypes appeared. Afterwards the isolated eastern populations rejoined the rest of the Mediterranean and the Atlantic. Additional sequencing of western Mediterranean samples is necessary in order to test whether there is a barrier to migration from the eastern to the western Mediterranean or if the transition area is located near the Gibraltar Straits.

Finally we have investigated the role of natural selection in shaping genetic variation in the carpet-shell clam. Tajima's tests [10] showed negative but non-significant values in all the markers, with the exception of marker CL102iA. This locus showed a value of D = +1.35. Although it was not significant, it suggests that this locus, which showed a high overall genetic differentiation but a low regional differentiation for RFLPs (Fig. 4), might be affected by selection.

References:

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Acknowledgements:

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